

## Original papers

# Fungi of the genus *Rhodotorula* isolated from the oral cavity of oncologic patients with colorectal cancer

Piotr Troska<sup>1</sup>, Ewa Sucharzewska<sup>2</sup>, Maria Dynowska<sup>2</sup>, Elżbieta Ejdys<sup>2</sup>

<sup>1</sup>Department of General Surgery, District Hospital, ul. Bartoszycka 3, 11-100 Lidzbark Warmiński, Poland

<sup>2</sup>Department of Mycology, University of Warmia and Mazury, ul. Oczapowskiego 1A, 10-719 Olsztyn, Poland

Corresponding Author: Ewa Sucharzewska; e-mail: ewko@uwm.edu.pl

**ABSTRACT.** This paper concerns the frequency of occurrence and enzymatic activity of fungi of the genus *Rhodotorula* isolated from the oral cavity of patients with colorectal cancer. Swabs from the oral cavity were subjected to standard mycological diagnostic procedures; enzymatic activity of the fungi was evaluated using an API Zym test by BioMérieux. The fungi of the genus *Rhodotorula* were noted in 22.5% positive isolates originating from women and 25.7% positive isolates originating from men. They included mono-species isolates of: *Rh. glutinis*, *Rh. minuta*, *Rh. mucilaginosa* and two-species isolates: *Rh. minuta* + *Candida dubliniensis*, *Rh. mucilaginosa* + *C. albicans* and *Rh. mucilaginosa* + *Saccharomyces cerevisiae*. The enzymatic activity of the isolated fungi was evaluated as high and very high in the case of acidic and alkaline phosphatase, esterase lipase, lipase, as well as leucine, valine and cysteine arylamidase. The highest values were determined in *Rh. mucilaginosa* with co-occurrence of *C. albicans* and *Rh. minuta* with co-occurrence of *C. dubliniensis*. In the ontosphere of oncologic patients, the increasingly frequent appearance of the species of fungi with high and very high enzymatic activity indicates the progressing risk posed by opportunistic forms, which includes *Rhodotorula* species.

**Key words:** *Rhodotorula*, oral cavity, enzymes, colorectal cancer

## Introduction

In oncologic patients, the oral cavity is colonized by fungi almost three times more frequently than in the normal subjects [1]. Lesions induced by fungi, mainly by yeast of different taxa, may be manifested as local infections of mucous membranes at the entries of infection or may lead to the development of systemic lesions afflicting the gastrointestinal tract, and even to fungemia [2,3].

The *Rhodotorula* genus is a polyphyletic taxon, representing anamorphic basidiomycetous yeasts from two subtypes: Pucciniomycotina and Ustilaginomycotina [4]. Fungi of this genus are included among the so-called “pink yeasts”; they prefer environments rich in organic matter of various origins, and exhibit a highly dynamic biocological activity. Most of species are saprotrophic endemics associated with the phyllosphere of subtropical and Mediterranean flora. They were isolated from the bioaerosole of air

[5], humid soil, fresh and saline waters [6,7], and even from drinking waters [8], from shrimps, fish and larvae of some insects [7], as well as from food products, fruits and fruit juices [9]. These fungi were reported to exhibit an especial metabolic activity in strongly eutrophicated waters and waters severely polluted with municipal sewage, which is indicative of their association with a human body [6,10,11]. Hence, some literature works suggest the applicability of some *Rhodotorula* species as indicators of fecal contaminations [6] and epidemiological risk [8].

The clinical significance of a few *Rhodotorula* species – typical opportunists: *Rhodotorula glutinis*, *Rh. minuta*, *Rh. mucilaginosa*, has been documented in the central Europe [6,9,12,13]. In Poland they are hardly ever addressed in medical parasitology and mycology. Baran [9] as well as Kurnatowska and Kurnatowski [12] have noticed that these fungi may asymptotically colonize the skin and mucous membranes of the oral cavity of the gastrointestinal

tract, vagina, and urinary ducts, and that in the immunosuppressed patients they may induce organ and systemic rhodotoruloses [13].

Results of longitudinal studies conducted at the Department of Mycology of the Warmia and Mazury University in Olsztyn, in cooperation with clinicians, show explicitly the increased expansiveness of some yeast strains in a human body. In the ontosphere, and particularly in the gastrointestinal tract of oncologic patients [11,14], there appear so far unnoted species [11,14] or species regarded as poorly pathogenic [11,13–15]. Considering study results which indicate the progressing risk posed by opportunistic species, an attempt was undertaken to determine the contribution of fungi from the genus *Rhodotorula* in positive mycological samples originating from the oral cavity of patients with colorectal cancer, considering the enzymatic activity of the isolated fungi.

## Materials and Methods

The experimental material included swabs from the oral cavity of 92 patients (42 women and 50 men) suffering from colorectal cancer confirmed in histopathological examinations. Biological specimens were subjected to standard mycological diagnostic procedures [12,14]. Initial macrocultures were incubated in a liquid Sabouraud's medium with the addition of gentamycin (0.025%) and chloramphenicol (0.1%) at temperatures of 25°C and 37°C, for 14 days. Once fungi growth had been observed, the material was plated onto a solid Sabouraud's medium with the same antibiotics and incubated at 37°C for 48h. The isolates were used to establish microcolonies on micro slides placed in a humid chamber. The microcolonies were grown on

Nickerson's agar enriched with a few drops of a broth with serum (1:1). To provide microaerophilic conditions, the whole culture was protected with a cover glass and incubated at 37°C for 48h and then at 25°C for 24h.

Species identification was based on their macroscopic, microscopic, and biochemical traits (API 20C, API AUX tests by BioMerieux). Taxonomic position of the isolated fungi was established using works by: Cooper [10], De Hoog et al. [5], Kurtzman et al. [7], and Krzyściak et al. [13].

Enzymatic activity of the fungi was evaluated using an API ZYM test by BioMerieux, which represents a semi-qualitative micro-method enabling determination of 19 hydrolytic exoenzymes. The enzymatic activity was expressed in nanomoles (nmol) of the hydrolyzed substrate according to the intensity of the color reaction, using a 5-scale according to the producer's recommendations: 0–0 nanomoles; 1–5 nanomoles, 2–10 nanomoles, 3–20 nanomoles, 4–30 nanomoles, and 5–>40 nanomoles.

## Results

Fungi were detected in 75 samples (81% of the analyzed patients), of which 40 isolates belonged to women (95%) and 35 isolates to men (70%). Fungi of the genus *Rhodotorula* were noted in 9 isolates originating from women (22.5%) and in 9 isolates originating from men (25.7%). They included mono-species isolates of: *Rh. glutinis*, *Rh. minuta*, *Rh. mucilaginosa*, and two-species isolates of: *Rh. minuta* + *Candida dubliniensis*, *Rh. mucilaginosa* + *C. albicans*, and *Rh. mucilaginosa* + *Saccharomyces cerevisiae* (Table 1).

The analysis of the enzymatic activity of

Table 1. Number of isolates with species from the genus *Rhodotorula* obtained from the oral cavity of patients with colorectal cancer

	Fungi species	Oral cavity	
		F	M
I	<i>Rhodotorula glutinis</i>	–	2
II	<i>Rhodotorula minuta</i>	1	1
III	<i>Rhodotorula mucilaginosa</i>	2	1
IV	<i>Rhodotorula minuta</i> + <i>Candida dubliniensis</i>	2	1
V	<i>Rhodotorula mucilaginosa</i> + <i>Candida albicans</i>	3	2
VI	<i>Rhodotorula mucilaginosa</i> + <i>Saccharomyces cerevisiae</i>	1	2

Table 2. Enzymatic activity of *Rhodotorula* spp. in individual isolates expressed in a 5-point scale – mean values

Number of enzyme	Enzyme	Enzymatic activity/the number of isolate					
		I	II	III	IV	V	VI
E2	Alkaline phosphatase (ALP)	3	3	3	4	5	2
E3	Esterase (C4)	3	2	3	4	4	2
E4	Esterase lipase (EL)	3	2	4	5	5	2
E5	Lipase (C14)	3	2	4	5	5	2
E6	Acrylamidase leucine	3	3	2	4	4	1
E7	Acrylamidase valine	3	3	2	4	4	2
E8	Acrylamidase cysteine	3	3	2	4	4	1
E9	Trypsin	0	0	0	2	2	1
E10	$\alpha$ -chymotrypsin	0	0	0	2	2	1
E11	Acid phosphatase	3	3	4	5	5	2
E12	Phosphohydrolase	2	2	3	4	4	1
E13	$\alpha$ -galactosidase	1	1	1	2	2	1
E14	$\beta$ -galactosidase	1	1	1	2	2	1
E15	$\beta$ glucuronidase	1	1	1	2	2	1
E16	$\alpha$ -glucosidase	2	1	2	3	3	1
E17	$\beta$ -glucosidase	2	1	2	3	3	1
E18	N-acetyl- $\beta$ -glucosaminidase	0	0	0	0	0	0
E19	$\alpha$ -mannosidase	0	0	0	0	0	0
E20	$\alpha$ -fucosidase	0	0	0	0	0	0

individual fungi species demonstrated that fungi from the genus *Rhodotorula* were capable of producing majority of the analyzed hydrolases (from 14 to 17). The enzymatic activity of the isolated fungi was evaluated as high and very high in the case of acidic and alkaline phosphatase, esterase lipase, lipase, as well as leucine, valine and cysteine acrylamidase. The highest values were determined in *Rh. mucilaginoso* with co-occurrence of *C. albicans* and in *Rh. minuta* with co-occurrence of *C. dubliniensis*. Noteworthy is a significantly lower enzymatic activity of *Rh. mucilaginoso* in isolates with *S. cerevisiae* (Table 2).

Very distinct differences were observed in enzymatic potencies of the discussed fungi between the mono-species isolates and the two-species isolates with *C. albicans* and *C. dubliniensis* – the same *Rhodotorula* species exhibited a higher enzymatic activity in the two-species than in the mono-species isolate. It applies not only to the aforementioned enzymes, but also to phosphohydrolase (a two-fold higher activity in the two-species isolates) as well as trypsin and  $\alpha$ -chymotrypsin that were not detected in isolates I, II and III, but were synthesized in isolates IV and V (Table 2).

## Discussion

Oncologic patients constitute a group at high risk of mycotic infections. Changes in the endogenous mycobiota, leading to disbacteriosis, as well as immunity suppression by the main disease and accompanying therapy, neutropenia, and catheterization provide excellent conditions for the proliferation of fungi, their enhanced trophic activity, and activation of their pathogenic potential [2,14,16,17]. It pertains mainly to the opportunistic forms – commensals of mucous membranes which at the moment of disturbing the biological homeostasis of a microorganism and disrupting its defense mechanisms undergo transition from saprotrophs into parasites [11,14]. It needs to be emphasized that usually patients become infected with their own strain. Hence, it is believed that mycological analyses of these ontocenoses of oncologic patients that have a direct contact with the environment (respiratory and digestive route) and which could be colonized by fungi even before the diseases develops are strongly recommended. One of these ontocenoses is the oral cavity which constitutes an ecological niche with the greatest

microbiological diversity, wherein fate of numerous potentially-pathological fungi is being decided. In extreme cases, yeasts from various taxonomic groups were detected in this ontocenosis even in 96% of the examined subjects [18]. The oral cavity may not only be a reservoir of potential anthropogenes, but also a route of mycotic invasion or spread. Oncologic patients are characterized by a high incidence of mixed infections [2,11,16,19], which was also confirmed in our analyses. The available literature addresses mainly the genera: *Candida*, *Geotrichum*, *Cryptococcus* or *Trichosporon* [1,2,11,14,15]. Looking at the results obtained in our study, however, it seems that in the case of oncologic patients the list of the most frequently isolated yeast should be completed with the genus *Rhodotorula*. It is also supported by the fact that some species of this genus are believed to be permanent components of the gastrointestinal tract and causative agents of severe infections in patients with immunosuppression, mainly the oncologic ones as well as parenterally nourished patients and HIV-positive ones [2,9,14,17,20,21]. These species include *Rhodotorula glutinis*, *Rh. minuta* and *Rh. mucilaginosa* – all were isolated in the presented study, but *Rh. mucilaginosa* was the most often isolated one. It was noted individually and together with *C. albicans* and *S. cerevisiae*. Cutrona et al. [22] claim *Rh. mucilaginosa* to be the most strongly associated with man, mainly with the gastrointestinal tract and skin [5,7]. Some authors believe this fungus to be a cosmopolitan species [13], most frequently inducing endogenous systemic infections in predisposed patients: cerebrospinal meningitis, endocarditis, peritonitis linked with peritoneal dialysis [13], fungemia in patients with AIDS [20], in patients with neutropenia and catheterized ones [5,7,13,21,23], and even these with onychomycosis [13]. Known are also cases of hospital infections induced by *Rh. mucilaginosa* [5].

The second, most frequently noted species turned out to be *Rh. minuta* – regarded by some authors as a commensal of the gastrointestinal tract of domestic animals. It was also noted in specimens from hairy skin of man together with keratinophilic fungi as well as in urine, feces and bronchoscopic fluid [5,7,9]. It is often a companion species to *C. albicans* [24]. Clinical cases induced by *Rh. minuta* include: postoperative panophthalmia [13,24], coxitis [13,22], systemic infections in patients with leukemia [5,13] and fungemia in catheterized

patients with AIDS.

The third species isolated only from the oral cavity of men was *Rh. glutinis*, classified by some authors as a low-pathogenicity saprotroph [13], which is rather disputable considering results of its enzymatic activity and some clinical data according to which it has been reported as an etiological factor of: keratitis and dacryoadenitis [5,7,13,16], cerebral meningitis [16,25,26], post-operative endocarditis [9,13], fungemia in catheterized patients [5,7,13,22,27] and patients after organ transplantation [24]. Alarming seems to be the isolation of the perfect stage of *Rh. glutinis* = *Rhodospidium diobovatum* from various sections of the alimentary tract of oncologic patients [11]. It shows the likely sexual reproduction of yeast in a human body and is indicative of the progressing adaptation processes that result from high vital strength and ecophysiological flexibility of the investigated fungi.

Baran [9] underscores also the similarity of allergenic properties of fungi from the genus *Rhodotorula* and these from the genus *Candida*, and indicates that hypersensitivity increases when these genera co-occur. Allergy to yeast may be manifested through chronic urticaria and may aggravate allergic rhinitis and to a lesser extent – asthma [9].

In the oncologic patients, rhodotorulosis may initiate an organ infection [17]. The reported massive colonization of the oral cavity mucosa is indicative of infection spreading into the lower sections of the gastrointestinal tract: esophagus and intestines [1,11,14,18].

Tissue colonization by fungi is facilitated by hydrolytic enzymes which by their chemical and physical effects on the environment ensure fungi persistence in tissues and participate directly in the digestion process of host proteins [12]. Many authors emphasize that the enzymatic activity, the proteolytic and lipolytic ones in particular, is indicative of the degree of pathogenicity, whereas in the opportunistic forms it additionally determines the rate of saprotrophism-to-parasitism transition. It needs to be emphasized that in the case of the analyzed *Rhodotorula* isolates, the proteolytic and lipolytic activity were high and very high, and that in the isolates co-occurring with *C. albicans* and *C. dubliniensis* it was even 2-fold higher. Acidic phosphatase, phosphohydrolase as well as esterase lipase and lipase reached the highest activity values, most likely as a result of either competition or synergy. Noteworthy is also that leucine, valine and

cysteine acrylamidase – as representatives of peptidases – are also capable of degrading components of epithelium (collagen) and factors taking part in immunological responses. Proteolytic enzymes determine the maintenance of the balance in the fungus-host interaction during body response to an infection and during infection development, and also affect the potential spread of the fungi [17]. By this means, they facilitate adherence and invasion, may affect the immune system of host and initiate inflammatory states, thereby enabling development of the pathological process. At the initial stages of infection, the activity of lipases is of major concern.

The exceptional enzymatic capabilities of fungi from the genus *Rhodotorula* have been noted and highlighted in hydromycological studies addressing waters contaminated with organic sewage [6]. The increased content of organic matter was accompanied by abundant growth of *Rh. glutinis* and *Rh. mucilaginosa* and by significant acceleration and enhancement of the metabolic activity with simultaneous reduction in bacterial counts. If similar correlations do occur in oral cavity microbiota, it may result in the eradication of bacteria that play the protective role and determine eubacteriosis. Taking into account the enzymatic activities determined in this study, we may speculate that fungi of the genus *Rhodotorula* are able to effectively compete also with other fungi.

## Conclusions

In the ontosphere of oncologic patients, the increasingly frequent appearance of the species of fungi with high and very high enzymatic activity indicates the progressing risk posed by opportunistic forms, which includes *Rhodotorula* species.

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